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SURVIVAL OF CHINESE HAMSTER OVARY CELLS FOLLOWING ULTRAHIGH DOSE RATE ELECTRON AND BREMSSTRAHLUNG RADIATION

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The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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The objective of this research was to measure cellular effects of ultrahigh dose rate X rays associated with high-power microwave devices. The intent was to detect differences in effect of ultrahigh dose-rate X rays compared to conventional dose-rate X rays at equivalent total doses. Cell survivability was used as the measure. No differences were noted until a dose of 4 Gray or greater was achieved.

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SURVIVAL OF CHINESE HAMSTER OVARY CELLS FOLLOWING ULTRAHIGH DOSE RATE ELECTRON AND BREMSSTRAHLUNG RADIATION

INTRODUCTION

For ionizing radiation effects, the dose rate at which the radiation is administered can have a dramatic effect on the observed biological consequences. The effect is most pronounced over the range 0.01 to 1 Gy/min, with increasing biological effects observed with increasing dose rate (1). At dose rates greater than 1.0 Gy/min, the biological response appears to be constant until ultrahigh dose rates are achieved. For the purpose of this report, ultrahigh dose rates are defined as dose rates in excess of 10^4 Gy/min.

A variety of studies have been conducted using pulsed electron radiation in nanosecond (ns) to microsecond (μs) pulses (2-4). In earlier studies (5 and 6), the observed decrease in biological effect of ultrahigh dose X rays was hypothesized to be a result of oxygen consumption; i.e., during the radiation exposure, the decrease in available oxygen was more rapid than oxygen diffusion to the irradiated volume, with a resultant hypoxia. Michaels et al. (2) observed no difference in survival of CHO cells exposed to 600 kV electrons (3 ns pulses) and ^{60}Co γ-irradiation under aerated conditions, whereas Schulz and co-workers (4) observed that high energy (30 MeV) pulsed electrons (10 ns pulses) were biologically less effective than 250 kVp X rays. There was no apparent difference in repair of sublethal radiation damage at isosurvival levels for CHO cells exposed to 600 kV electrons and 280 kVp X rays (3).

The TEMPO unit at Kirtland Air Force Base, New Mexico, is a high-power microwave source. During operation of the unit, low energy X rays are produced during pulses which are of extremely short duration (120-140 ns); the X rays, therefore, are delivered at an ultrahigh dose rate per pulse. At a high repetition rate, the potential is present for delivery of an ultrahigh dose rate per (more conventional) unit of time. This study was undertaken to determine if there was any increased risk to personnel exposed to ultrahigh dose rate irradiation. Preliminary studies were conducted on-site at the TEMPO unit to measure the production of chromosome aberrations following high dose rate, low total dose X rays (7). Whereas, they showed that the X rays did produce chromosome aberrations, it was not determined if conventional dose rates will induce a similar response. The TEMPO unit can only be operated, however, at a pulse repetition frequency of 10 pulses/minute. This frequency rate would result in an average dose rate (over time) of approximately 5-6 cGy/min. To avoid potential confounding variables of such a low average dose rate, approval was obtained to conduct studies using the linear accelerator (LINAC) at the Armed Forces Radiobiology Research Institute (AFRRI) in Bethesda, Maryland. The LINAC can be used for either pulsed electron or bremsstrahlung (X ray) exposures, and has a variable repetition rate (8). In performing these studies, consideration must be given to the fact that the X rays produced by the TEMPO unit are of low energy, while those of the available sources would be of higher energies and, therefore can be expected to have different relative biological effectiveness (RBE). Gerweck et al. showed

that 50 kVp X rays are more efficient for cell killing than ^{137}Cs γ-rays (9), ^{60}Co γ-rays (2), or 280 kVp X rays (3).

MATERIALS AND METHODS

Cell Culture

Exponentially growing cultures of Chinese hamster ovary (CHO) cells were maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum and 40 μg/ml gentamycin. Stock cultures were incubated at 37 °C in a humidified 5% CO_2/95% air incubator. In preparation for conducting experiments with the LINAC at the AFRRI, 75-cm^2 tissue culture flasks of exponentially growing cells were filled with prewarmed medium at the University of Texas Health Science Center at San Antonio (UTHSCSA) and placed in a portable cooler with prewarmed insulators. These flasks were kept in a cooler for approximately 8 h during transportation to the AFRRI, where they were placed into a 37 °C incubator until they were to be distributed for experiments.

Cell Survival

The cells were detached from the stock culture flasks using a standard trypsinization technique, counted by hemocytometer, and plated into 25-cm^2 tissue culture flasks 18 h before radiation exposure. Six to eight replicate flasks were used for each dose (at varying cell densities). Additional flasks were seeded with 5000 cells for multiplicity determination. Immediately before radiation, the attachment medium in each flask was aspirated, and the flasks were completely filled with prewarmed (37 °C) medium. The cap and neck of the flasks were wrapped with Parafilm to prevent leakage. Flasks were irradiated in a vertical position in a specially constructed holder (Fig. 1). The surface with attached cells was facing the radiation source, and 1.5-3.0 cm of polystyrene was placed between the flasks and the LINAC as build-up material (Fig. 2). Following exposure, the medium in the flasks was decanted and replaced with 5 ml of fresh prewarmed medium. The flasks were again returned to the 37 °C incubator, where they remained undisturbed for 7 days to allow for colony formation. Colonies were fixed and stained with crystal violet prepared in 95% ethanol. To determine cellular multiplicity, 2 additional flasks of cells were fixed with 3:1 methanol:glacial acidic acid at the beginning and end of treatment; the multiplicity was calculated as the average number of cells per colony forming unit. The surviving fraction was corrected for plating efficiency (80-90%) and cellular multiplicity at the time of treatment.

The cell survival was averaged for 3 independent experiments, performed 3-4 weeks apart. The standard error of the mean is indicated for each datum point (when larger than the datum point). The exponential portions of the survival curves were fitted by linear regression; the points in the shoulder region were fitted by eye.

Radiation Treatments

For continuous irradiation studies, the ^{137}Cs Gammacell-40 source at the UTHSCSA was used. Flasks with attached cells were filled with prewarmed medium and capped tightly before being placed in a horizontal position in the irradiator. The Gammacell-40, a bilateral radiation source, operated at a dose

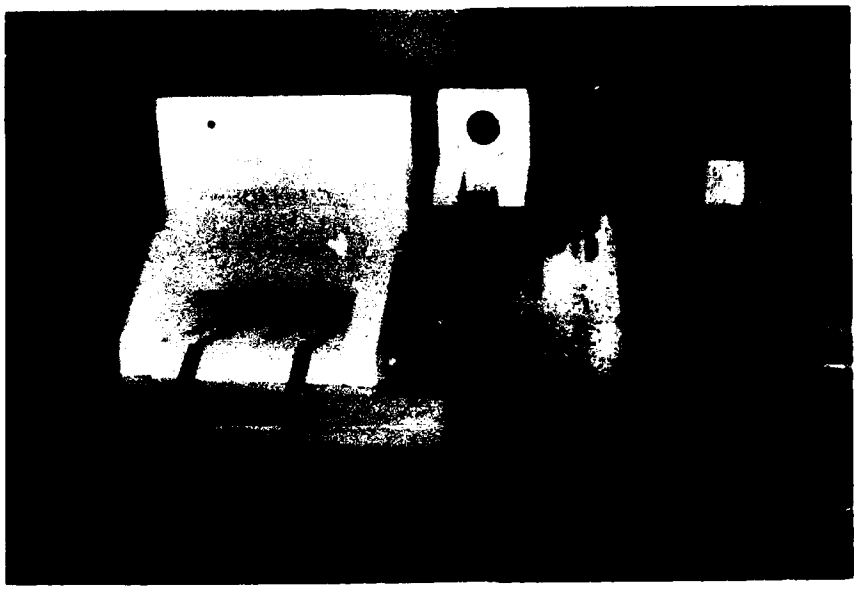

Figure 1. Linear accelerator facility at the AFRRI.

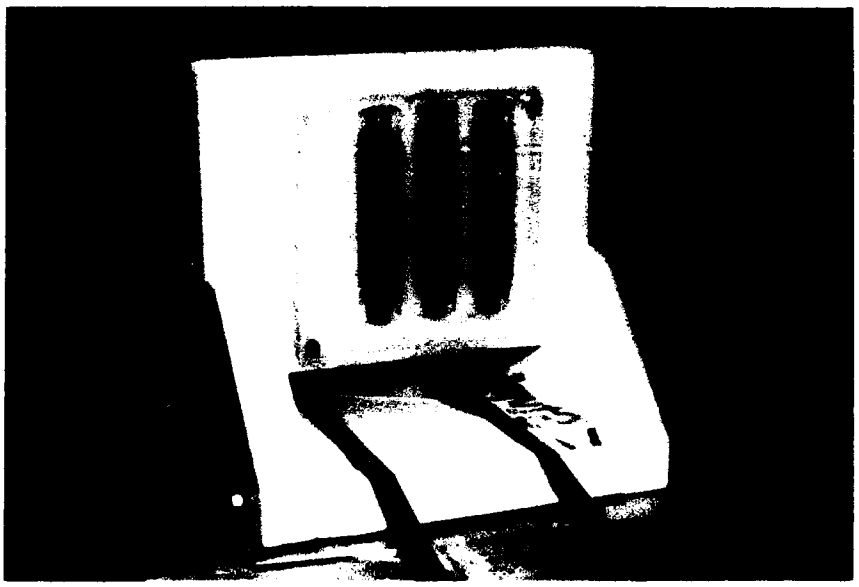

Figure 2. Irradiation set-up for exposure of CHO cells to either electrons or bremsstrahlung produced by the LINAC. The flasks are in a vertical orientation behind polystyrene material.

rate of 1.24 Gy/min. A second series of experiments was conducted at low dose rates in the same Gammacell-40 unit; a lead attenuator was placed in the irradiator with a resultant dose rate of 0.23 Gy/min.

Experiments with pulsed radiation were conducted at the AFRRI using the LINAC as a source of both high-energy electrons and bremsstrahlung (X rays) (as described in reference 8). The latter were produced using a water-cooled tantalum bremsstrahlung converter with 1.7-mm lead hardening filter and a stepped lead flattening filter. The electron energy used for these studies was 13 MeV. Dosimetry was performed by the Health Physics Division of AFFRI using thermoluminiscent dosimeter (TLD) chips at the beginning and end of each day. The TLD chips were precalibrated and the dose was calculated per pulse of radiation. In all cases, the duration of the pulses was 300 ns. This duration was the minimum pulse width for which beam quality was consistent. Four different radiation exposure conditions were studied:

1. Ultrahigh dose-rate electrons - 30 pulses/second, 300 ns pulses.
2. Conventional dose-rate electrons - 3 pulses/minute, 300 ns pulses.
3. Conventional dose - rate bremsstrahlung - 30 pulses/second, 300 ns pulses.
4. Low dose-rate bremsstrahlung - 7.5 pulses/second, 300 ns pulses.

The dose per pulse of electrons varied from 27.35 to 32.95 cGy/pulse, while that for bremsstrahlung varied from 0.046 to 0.050 cGy/pulse for the 3 experiments. The maximum pulse repetition rate that could be obtained was 30 pulses/second (1,800 pulses/minute), which resulted in a maximum (average) dose rate of approximately 550 Gy/min for electrons and 0.86 Gy/min for bremsstrahlung. The pulse repetition rate was also lowered, so that a comparable dose rate could be obtained with electrons (condition 2) as for the bremsstrahlung (condition 3). The last exposure condition (condition 4) was examined to determine if a low-dose-rate effect was present for pulsed radiation. Although the average dose rate that was actually obtained for electrons was 550 Gy/min, if calculated for each 300 ns pulse, the dose rate per pulse was 6×10^{7} Gy/min. For the bremsstrahlung, the dose rate per pulse was 9.6×10^{4} Gy/min (Table 1).

RESULTS

The radiation responses of CHO cells exposed to ^{137}Cs are shown in Figure 3. When cells were irradiated at low dose-rate (0.23 Gy/min) there was less cell killing than at conventional dose rate (1.2 Gy/min). The values for D_o and D_q were calculated and are shown in Table 1.

TABLE 1. SURVIVAL CURVE PARAMETERS FOR CHO CELLS EXPOSED TO ^{137}Cs γ-RAYS OR PULSED ELECTRONS OF X RAYS AT DIFFERENT DOSE RATES

Radiation	Dose/ pulse (cGy/pulse)	Pulse repetition rate	Actual dose rate/ pulse (Gy/min/pulse)	Average dose-rate (Gy/min)	Survival curve parameters D_o(Gy)	D_q(Gy)
^{137}Cs	-	-		1.24	1.8	1.2
	-	-		0.23	2.2	0.45
electrons (LINAC)	~30	30/s	$6x10^7$	550	1.1	2.45
	~30	3/min	$6x10^7$	0.90	1.2	2.55
bremsstrahlung (LINAC)	~0.5	30/s	$9.6x10^4$	0.90	1.2	2.55
	~0.5	7.5/s	$9.6x10^4$	0.21	1.55	1.85

The CHO response to pulsed radiation from the LINAC is shown in Figure 4. These data are the average of 3 separate experiments. The greatest cell killing is observed for the ultrahigh dose-rate electrons, delivered at a high pulse repetition frequency, and a resultant high average dose rate (550 Gy/min). At a lower pulse repetition rate, the survival is higher. There is no difference in survival for equivalent average dose rates of electrons delivered at this lower repetition frequency and a lower average dose rate (0.9 Gy/min), or bremsstrahlung (produced by those electrons) delivered at a high repetition rate, but the same average dose-rate (0.9 Gy/min). Finally, there is also a marked sparing effect if the pulsed bremsstrahlung radiation is administered at a low average dose rate (~21 cGy/min). There is no difference in the D_o value for the electrons at the highest average dose rate (550 Gy/min) compared with electrons at 90 cGy/min; however, at the lower dose rate, there is evidence of a slightly larger shoulder (Table 1). At the low average dose rate of bremsstrahlung, the D_o was increased by a factor of 1.3, and an obvious decrease in the shoulder (D_q) was observed.

Of interest was the observation that the cell survival following the lowest average dose rate obtained with the LINAC was less than that of the higher dose rate with continuous (^{137}Cs) radiation (compare Figs. 3 and 4). This observation is better illustrated in Figures 5 and 6, in which the survival for the different radiations and dose rates was compared after total exposure doses of 10 Gy (Fig. 5) or 12 Gy (Fig. 6). Cell survival after a total dose of 10 Gy was significantly lower following exposure to ultrahigh dose rate electrons at the maximum repetition rate obtained, than for the other exposure conditions (Fig. 5). The pulsed radiation from the LINAC, both electrons and bremsstrahlung, was more effective in terms of cell killing than the continuous exposures from ^{137}Cs γ-rays. This effect was even more pronounced at 12 Gy (Fig. 6). There was no significant difference in survival between pulsed electrons and X rays (bremsstrahlung) delivered at the same average dose rate (0.90 Gy/min).

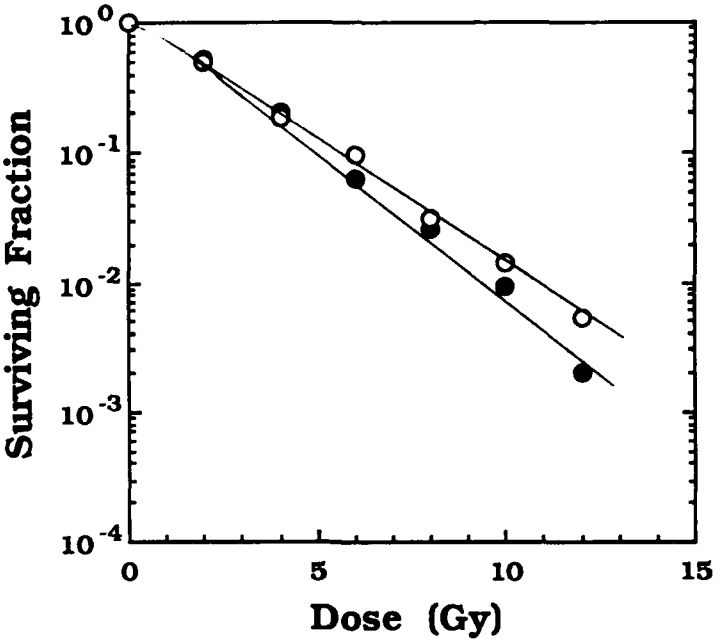

Figure 3. Survival following low dose rate or conventional dose rate ^{137}Cs radiation exposure. The CHO cells were irradiated at either 1.24 Gy/min (●) or 0.23 Gy/min (○). Standard errors are shown when larger than the data points.

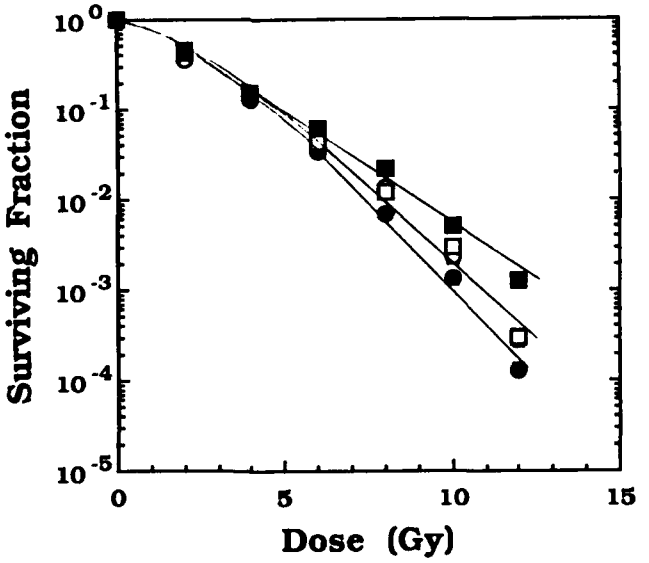

Figure 4. Survival of CHO cells following exposure to pulsed electrons or bremsstrahlung (X rays). Cells were irradiated with pulsed electrons (300 ns pulses) at a pulse repetition rate of either 30 pulses/s (●) or 3 pulses/min (○), or irradiated with bremsstrahlung (X rays) at a pulse repetition rate of either 30 pulses/s (□) or 7.5 pulses/s (■). See Table 1 for details of average dose rates and dose rate per pulse.

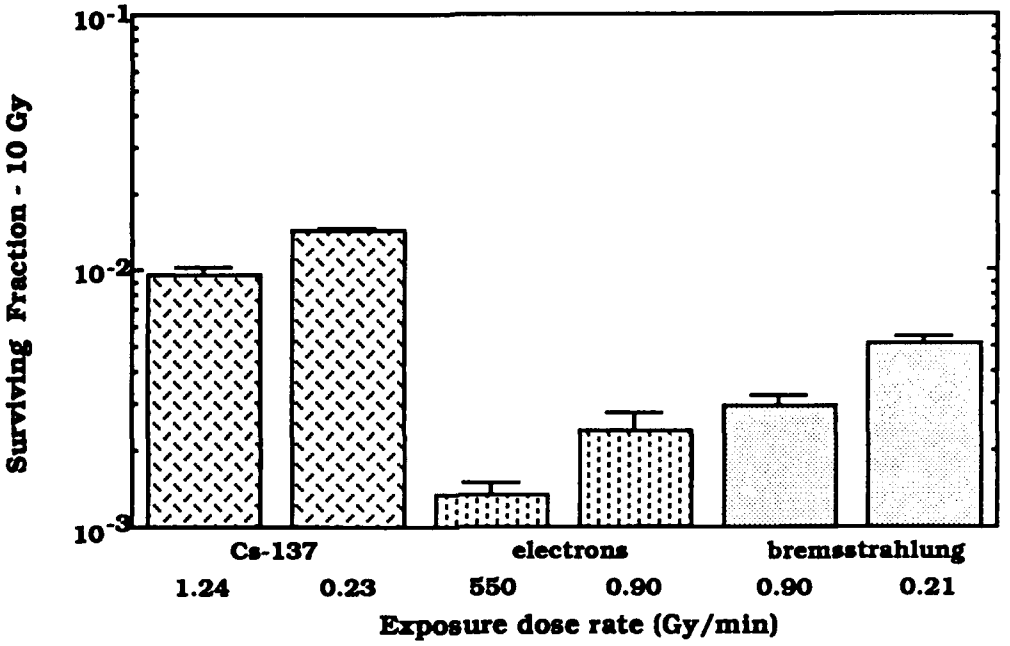

Figure 5. Survival of CHO cells following exposure to a total dose of 10 Gy. Cells were exposed to either continuous ^{137}Cs γ-rays or pulsed electrons or X rays at different dose rates.

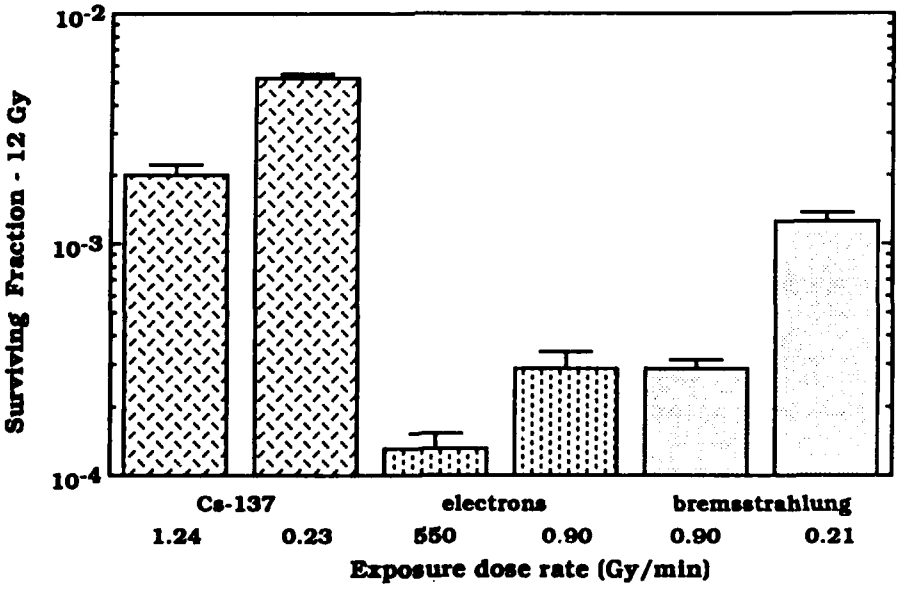

Figure 6. Survival of CHO cells following exposure to a total dose of 12 Gy. Cells were exposed to either continuous ^{137}Cs γ-rays or pulsed electrons or X rays at different dose rates.

DISCUSSION

The survival of CHO cells, after exposure to ultrahigh dose-rate electrons (high dose/second/pulse), when delivered at a high pulse repetition rate (550 Gy/min), was less, for the same total dose, than when delivered at a lower repetition rate, and therefore a lower average dose rate (0.9 Gy/min). Since a similar level of survival was obtained for CHO cells exposed to the bremsstrahlung (produced using the electrons at a high repetition rate) at the same lower average dose rate as for the lower repetition rate electrons, and since these exposures resulted in equivalent D_o and D_q values, the dose per pulse factor cannot explain the difference. It appears to be an average dose rate difference between 550 Gy and 0.9 Gy. It is important to note that these differences in survival were only obvious at higher total doses of radiation, i.e., greater than 4 Gy. Whereas other investigators have examined the effect of dose rate, there do not appear to be any other studies reported where an attempt has been made to compare the effect of pulse repetition rate (for pulsed radiation from the same sources) on cell survival. In one study which examined murine mortality following exposure to pulsed electrons at high and conventional average dose rates, it was demonstrated that an observed dose-rate effect was dependent on the endpoint. There was no effect of high dose-rate electrons (60 Gy/min) on the 30-day mortality of mice ($LD_{50/30}$) as compared to electrons delivered at 1 Gy/min, but the high dose-rate electrons were more effective for murine death within 4 days ($LD_{50/4}$) (10).

When the results for the continuous ^{137}Cs exposure (1.24 Gy/min) are compared with the pulsed electron and bremsstrahlung exposures at average dose rates of 0.9 Gy/min, it is interesting to note that both of the latter are more effective than the ^{137}Cs in killing cells, using either cell survival or the calculated D_o values as criterion. Even when the average dose rate of the pulsed bremsstrahlung is lowered to 0.21 Gy/min, and the expected increased survival due to a dose-rate effect is seen, the survival is still lower than after the 1.24 Gy/min ^{137}Cs exposure. For the ^{137}Cs, the lowering of the average dose rate from 1.24 Gy/min to 0.23 Gy/min results in the expected sparing effect, and the highest survival observed for a similar total dose by any exposure condition.

If the calculated D_o values (Table 1) and the actual cell survival for doses greater than 4 Gy are used as criteria, both pulsed electrons and pulsed X rays (bremsstrahlung) from the LINAC are more efficient in killing mammalian cells than continuous exposure to ^{137}Cesium γ-rays at moderate and lower dose rates. This finding appears to be in contrast to studies by other investigators, who have compared mammalian cell survival after exposure to ultrahigh dose rate, pulsed electrons (30 MeV) versus exposure to 280 kVp X rays; they reported that X rays were more effective for cell lethality (3,4). In the latter experiments (4), the average dose rate per electron pulse was 5×10^{9} - 5×10^{10} Gy/s/pulse, which is approximately 100-1,000 times greater than for the electrons in the experiments reported here. At these extremely high dose rates, oxygen depletion may have been, at least in part, responsible for the observed effect. We do not have a hypothesis for the reversed effect observed in our studies. A planned comparison of the relative biological effectiveness of ^{137}Cs γ-rays versus conventional X rays in the CHO cell survival system used in these investigations will add necessary information in attempting to explain the results observed. This information would allow a determination of the relative biological effectiveness of ^{137}Cs γ-rays in this system.

CONCLUSIONS

a) Ultrahigh dose-rate pulsed radiation (both electrons and bremsstrahlung), delivered at a high repetition rate, may present a greater risk, with respect to cell killing, than continuous radiation delivered at the same average dose rate;

b) This effect is not obvious, however, in the shoulder region of the survival curve, or at doses below 4 Gy. Doses must be greater than 2 Gy, for acute whole body exposures, to be lethal to at least some humans;

c) There is a sparing effect observed if the average dose rate is lowered, for any of the radiation modalities examined.

One factor we did not address in this study is the effect of beam quality. The TEMPO unit at Kirtland AFB is known to produce low-energy X rays. It has been shown that 50 kVp X rays have an increased relative biological effectiveness (RBE) for cell lethality compared with 280 kVp X rays (3), and that they are also more effective for cell killing than ^{137}Cs (9). The bremsstrahlung we studied here is of much higher energy, and it is unclear whether the low energy quality of the TEMPO X rays would change the relative survivals, especially in the low-dose region (< 2 Gy). The dose rate per pulse of the X rays produced by the TEMPO is intermediate (2.3×10^{6} Gy/min) between the electrons (6×10^{7} Gy/min) and the bremsstrahlung (9.6×10^{4} Gy/min) available from the LINAC. If there is a factor related to the pulsing phenomenon, then the TEMPO unit X rays could be more effective than ^{137}Cs γ-rays. However, the extremely low repetition frequency at which the TEMPO unit can be operated would result in an extremely low average dose rate. It is obvious from our studies that the latter factor is of major importance, and may offset any possible increased effectiveness for cell killing by the X rays of the TEMPO unit due either to their pulsed nature, or their low quality. Further studies would be necessary to assess this possibility, as well as the potential genetic risk of ultrahigh dose-rate radiation at low doses.

REFERENCES

1. Hall, E.J. Radiation dose-rate: A factor of importance in radiobiology and radiotherapy. Br J Radiol 45:81-97 (1972).

2. Michaels, HB, E.R. Epp, C.C. Ling and E.C. Petersen. Oxygen sensitization of CHO cells at ultrahigh dose rates: Prelude to oxygen diffusion studies. Radiat Res 76:510-521 (1978).

3. Gerweck, L.E., E.R. Epp, H.B. Michaels, C.C. Ling and E.C. Petersen. Repair of sublethal damage in mammalian cells irradiated at ultrahigh dose rates. Radiat Res 77:156-169 (1979).

4. Schulz, R.J., R. Nath and J.R. Testa. The effects of ultrahigh dose rates on survival and sublethal repair in Chinese-hamster cells. Int J Radiat Biol 33:81-88 (1978).

5. Town, C.D. Effect of high dose rates on survival of mammalian cells. Nature 215:847-848 (1967).

6. Berry, R.J., E.J. Hall, D.W. Forster, T.H. Storr and M.S. Goodman. Survival of mammalian cells exposed to X rays at ultrahigh dose rates. Br J Radiol 42:102-107 (1969).

7. Ciaravino, V. and M.L. Meltz. Chromosome aberrations due to high-dose X-rays. Final report of contract No. C-4953 submitted to Quest Research Corp, 1988.

8. Gee, M.T. LINAC Facility at Armed Forces Radiobiology Research Institute. AFRRI Technical Report, TR84-3, Bethesda, Maryland 1984.

9. Bonura, T., D.A. Youngs and K.C. Smith. R.b.e. of 50 kVp X-rays and 660 keV γ-rays (^{137}Cs) with respect to the production of DNA damage, repair and cell-killing in Escherichia coli K-12. Int J Radiat Biol 28:539-548, (1975).

10. Hornsey, S. and T. Alper. Unexpected dose-rate effect in the killing of mice by radiation. Nature 210:212-213 (1966).

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